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Effects of Two Petroleum Products on *Pocillopora damicornis* Planulae[1]

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ABSTRACT: *Pocillopora damicornis* planulae were exposed to different concentrations of benzene and gasoline:oil mixtures to determine the lethal concentrations and biological responses of the coral larvae. Bioassay tests with either open or closed static solutions of the test compounds were monitored. Planulae settlement was considered as the visible reaction to the hydrocarbon compound introduced. This study found that corallite formation was significantly influenced by the different concentrations of the test compound, but no clear correlation between concentration of the test compound and rate of corallite formation was ascertained. Mortality was minimal in most of the test concentrations utilized in the experiments.

MARINE POLLUTION HAS BEEN defined as "... the introduction by man, directly or indirectly, of substances or energy into the marine environment (including estuaries) resulting in such deleterious effects as harm to living organisms, hazards to human health, hindrances to marine activities like fishing, impairment of the quality for use of seawater, and the reduction of amenities ..." (Gomez 1988). In this regard, numerous studies concerned with the effects of pollutants on living organisms have documented the effects of sediment, sewage, pesticides, heavy metals, and petroleum products (Smith et al. 1973, Anderson et al. 1974, Marsh and Gordon 1974, Anderson 1977, Johnson 1977, Lee 1977, Michael 1977, Rice et al. 1977, Sanborn 1977, Randall and Birkeland 1978, Malins 1979, Rinkevich and Loya 1979, Middleditch and Basile 1980, Baker 1981, Chansang 1988, Hodgson and Dixon 1988, Hungspreugs 1988, McManus 1988, Ford 1989, Klerks and Levinton 1989). However, most of these studies have concentrated on the use of certain organisms that were selected because of their availability, ease of handling, hardiness in the laboratory, and clarity of response to stress. Howarth (1989) and Levine (1989) have pointed out several drawbacks in the current convention of environmental testing and monitoring: (1) among species, certain groups are more tolerant to pollution stress than others; (2) within species, an organism may be more sensitive at one point of its life cycle than at another; (3) most of the experiments done in the past were of short duration (3–5 days), thereby causing underestimation of long-term sublethal effects.

Of the studies that have discussed the effects of pollutants on corals (Loya 1976, Olafson 1978, Rinkevich and Loya 1979, Baker 1981, Peters et al. 1981, Coles 1985, Esquivel 1986, Glynn et al. 1986, Howard and Crosby 1986, Hodgson and Dixon 1988, Hodgson 1990), few have dealt with the use of coral larvae as the study animals (Coles 1985, Esquivel 1986, Hodgson 1990). This may be due to the very sensitive nature of the coral larvae (Babcock 1985). Furthermore, there has been difficulty in monitoring the precise time of death of certain coral larvae (Esquivel 1986). However, it has been proposed that for the coral *Pocillopora damicornis* the larval stage may be the more resistant phase of the coral's life cycle (Edmondson 1946, Coles 1985, Esquivel 1986). If so, how resistant is the coral larva to environmental stress? How does the coral larva react when exposed to chemical pollutants? These are but a few questions that need to be answered by current

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environmental studies and coral reef research.

The main concerns of this study were (1) to determine the lethal and sublethal concentrations of two petroleum-based products on the longevity and viability of *P. damicornis* larvae (planulae); and (2) to describe the biological responses of coral planulae to the two test compounds. It is hoped that results from this study will help evaluate the suitability of coral larvae as bioassay tools for environmental monitoring schemes.

MATERIALS AND METHODS

Planulae Collection and Maintenance

Pocillopora damicornis planulae were gathered from six coral heads with an average diameter of 10 cm following the procedure of Richmond and Jokiel (1984). Coral heads were collected from reef no. 10, Kaneohe Bay, Hawaii, on 18 June 1989 (see Figure 1). Planulae were kept in 500-ml glass containers filled with unfiltered seawater. These containers were previously washed with phosphorus-free dishwashing liquid (Palmolive brand, unscented) before use. This procedure was found to be an effective way of keeping free-swimming planulae alive and did not have any detrimental effects on the health and viability of the planulae. Stocking density was maintained at 100 individuals per container to minimize stress from crowding and water fouling. Seawater was changed daily to maintain good water quality. Planulae used for the bioassays were randomly selected from each container regardless of spawning dates.

Bioassay with Gasoline:Oil Mixture

OPEN SYSTEM: Regular unleaded gasoline mixed with motor oil (SAE 40) at the standard motor boat engine dilution ratio of 50:1 was used to determine the potential effects of increased recreational water sports activities on coral planulae. Four different test concentrations were prepared via direct dilution of the test compound with unfiltered seawater (5, 10, 50, and 100 ppm). Three replicates of 15 planulae each per treatment were established in 50-ml plastic petri dishes. A fifth group with 15 planulae was established without the test compound to serve as controls. Plastic petri dishes were used and the potential effects of hydrocarbon interaction with the plastic material was not considered. All the petri dishes were covered and the whole experimental set-up was kept under shade to minimize temperature fluctuations and rapid evaporation of the test solution. Hourly observations of the planulae were made for the first 6 hr. Six-hour intervals were used for the next 3 days. A 12-hr sampling regime was adopted for the final 13 days of the bioassay period. Settlement of the planulae was used as the reaction parameter for this study.

CLOSED SYSTEM: To minimize the volatility of the gasoline:oil mixture, 200-ml wide-mouth clear glass bottles with aluminum foil–lined caps were used as bioassay containers. Four concentrations of the test compound (1, 5, 20, and 100 ppm) were prepared by direct dilution with three replicates of 15 planulae per container per concentration. The whole experimental group was placed in a shallow tub with running seawater to maintain ambient temperature as close to that of the environment as possible. Observation procedures similar to those used with the open system were followed. The test solutions were not changed for the duration of the experiment to minimize stress on the planulae being studied.

Bioassay with Benzene

CLOSED SYSTEM WITHOUT SETTLING PLATES: Benzene is one of the many hydrocarbon products from the natural breakdown of petroleum. This chemical has rapid inherent volatility such that no open systems were established. Experimental procedures similar to those used with the closed system of the previous group were followed. Exposure time was 10 days.

CLOSED SYSTEM WITH SETTLING PLATES: To determine if increased surface area for settlement had any influence on the reaction of the planulae toward the test compounds, four settling plates were added to the bioassay containers. These settling plates were glass

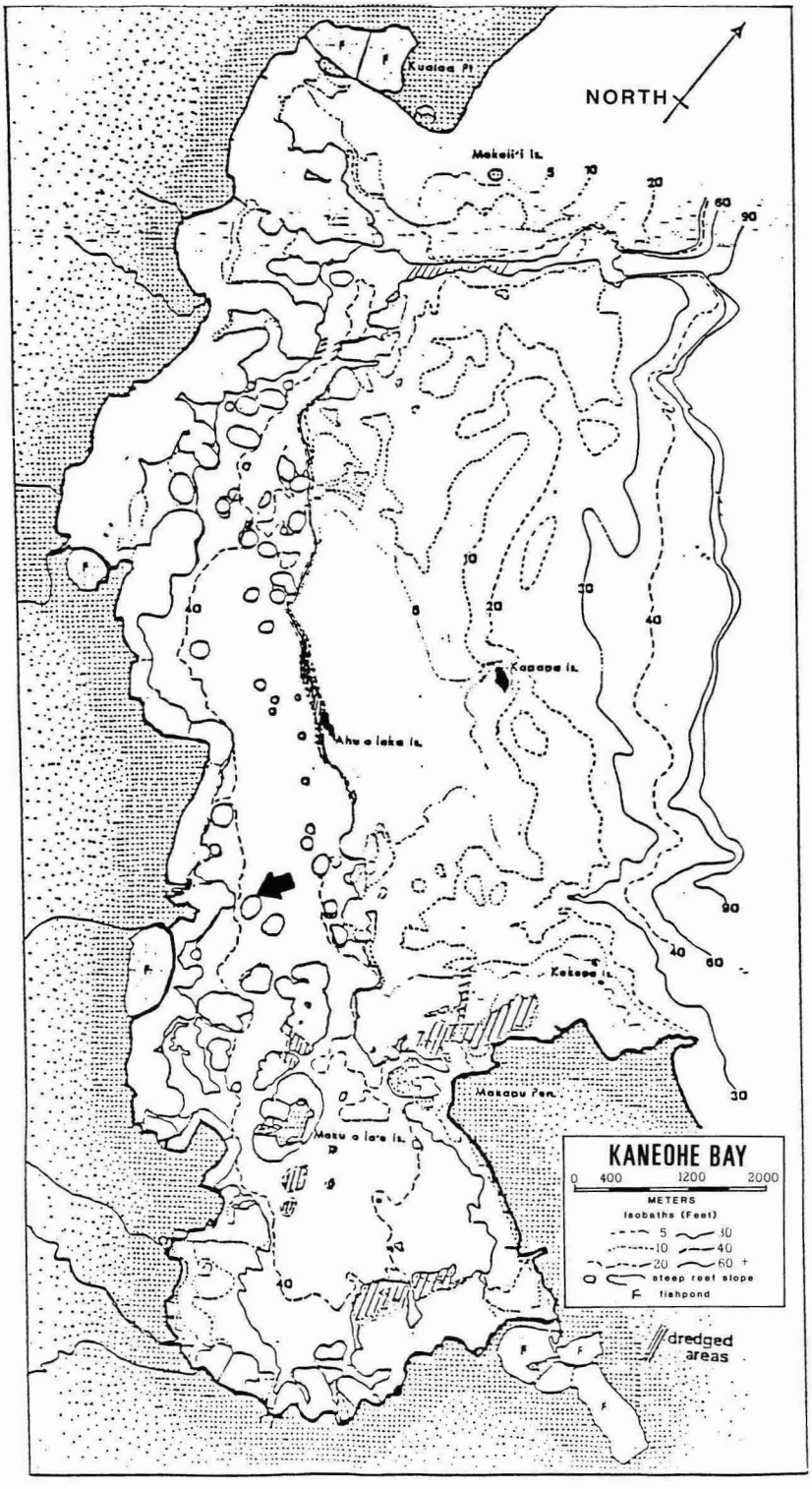

FIGURE 1. Map of Kaneohe Bay with arrow showing reef no. 10.

microscope slides left in running seawater for a few days. This procedure allowed the "conditioning" of the glass slides, resulting in the formation of a thin mucus film on the surface (Harrigan 1972). The same experimental procedures described above were followed. Exposure time was 6 days.

Multivariate analysis of variance (ANOVA) with repeated measures was undertaken using the "*4 V*" B M D P statistical software package.

RESULTS

Bioassay with Gasoline:Oil Mixture

OPEN SYSTEM: Unlike many organisms that readily show mortality or moribundity during toxicity bioassays even at very low concentrations, *P. damicornis* planulae seem to be resistant to the concentrations used in these experiments. Mortality was not noted, but planulae metamorphosed, calcified, and settled on the sides of the petri dishes in two of the treated containers (Figure 2). All of the other experimental treatments did not elicit settlement response. Multivariate ANOVA tests revealed significant treatment and time effects for this data set. This implies that the mean numbers of corallites per concentration were significantly different from one another and that the mean numbers of corallites also varied significantly per day. Variability between replicates was minimal (Table 1).

CLOSED SYSTEM: Most of the treatments in this experiment showed corallite formation after 3 days of exposure, with total mortality of the planulae observed for the 100-ppm group after 2 days (Figures 3 and 4). Multivariate ANOVA tests revealed significant

TABLE 1

EFFECT OF GASOLINE:OIL MIXTURE ON *Pocillopora damicornis* LARVAE KEPT IN 50-ML PETRI DISHES HELD IN AN OPEN SYSTEM

FACTOR	MEAN SQUARE	df	*P*
Number of corallites	15.51	1, 10	0.0013
Between treatments	10.69	4, 10	0.0005
Between days	1.10	12, 120	0.0000
Error	0.79		

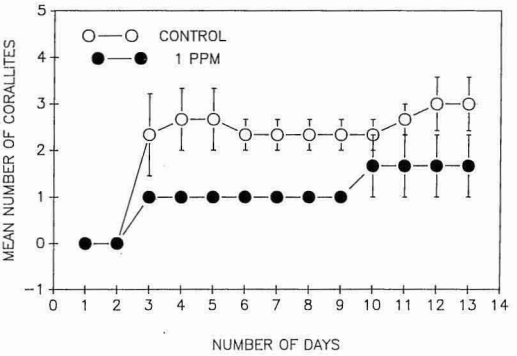

FIGURE 3. Effect of gasoline:oil mixture on planulae kept in a closed system (control and 1 ppm).

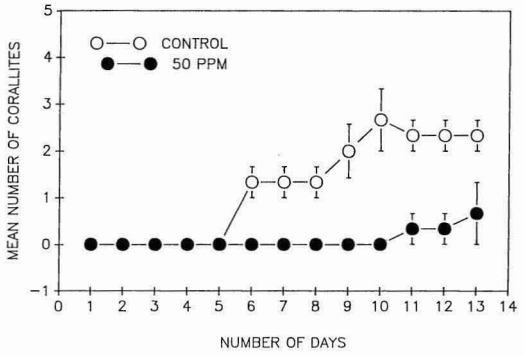

FIGURE 2. Effect of gasoline:oil mixture on planulae kept in an open system (control and 50 ppm).

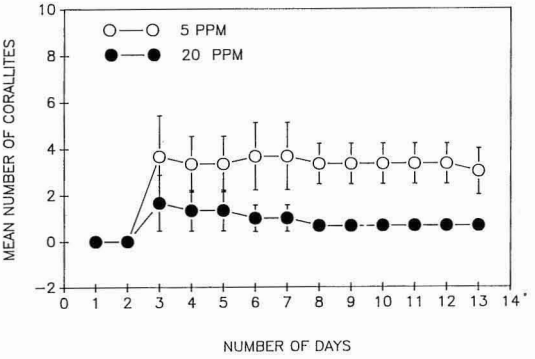

FIGURE 4. Effect of gasoline:oil mixture on planulae kept in a closed system (5 ppm and 20 ppm).

differences between treatments and between days (Table 2).

Bioassay with Benzene

CLOSED SYSTEM WITHOUT SETTLING PLATES: No mortality was recorded in these experiments, and no settlement was seen for the 1-ppm set (Figures 5 and 6). Statistical analyses of the planula settlement data revealed no significant differences between treatments, but noticeable differences were present between days (Table 3).

TABLE 2

EFFECT OF GASOLINE:OIL MIXTURE ON *Pocillopora damicornis* LARVAE KEPT IN 200-ML CONTAINERS HELD IN A CLOSED SYSTEM

FACTOR	MEAN SQUARE	df	*P*
Number of corallites	362.85	1, 10	0.0001
Between treatments	51.89	4, 10	0.0118
Between days	5.61	12, 120	0.0000
Error	9.09		

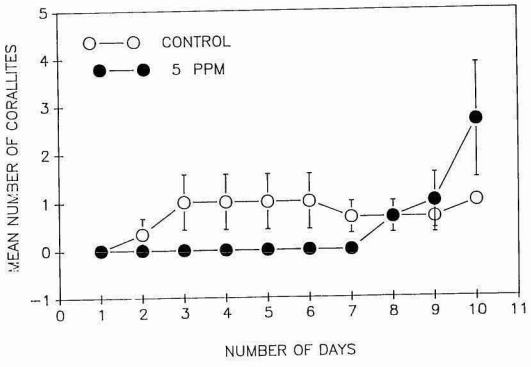

FIGURE 5. Effect of benzene on planulae kept in a closed system without plates (control and 5 ppm).

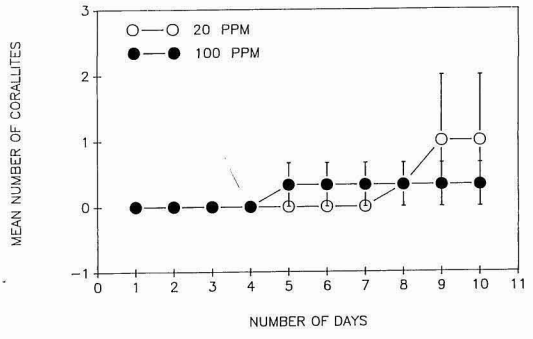

FIGURE 6. Effect of benzene on planulae kept in a closed system without plates (20 ppm and 100 ppm).

TABLE 3

EFFECT OF BENZENE ON *Pocillopora damicornis* LARVAE IN 200-ml CONTAINERS WITHOUT SETTLING PLATES HELD IN A CLOSED SYSTEM

FACTOR	MEAN SQUARE	df	*P*
Number of corallites	13.50	1, 10	0.0048
Between treatments	7.00	4, 10	0.2297 (n.s.)
Between days	1.41	9, 90	0.0000
Error	1.04		

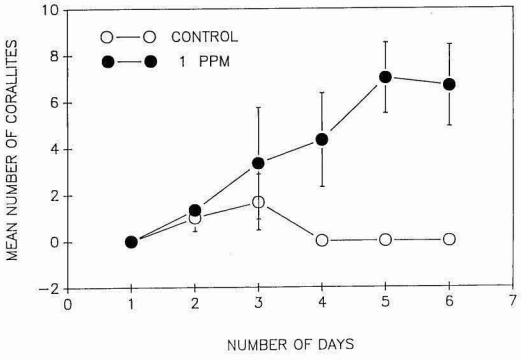

FIGURE 7. Effect of benzene on planulae kept in a closed system with plates (control and 1 ppm).

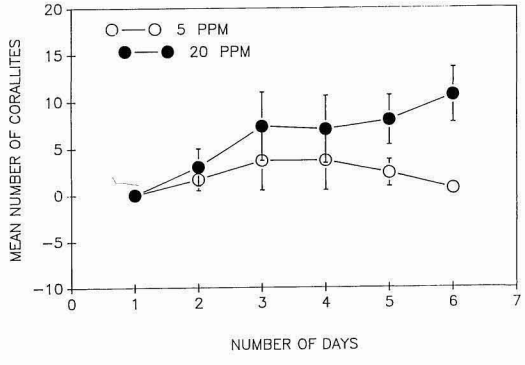

FIGURE 8. Effect of benzene on planulae kept in a closed system with plates (5 ppm and 20 ppm

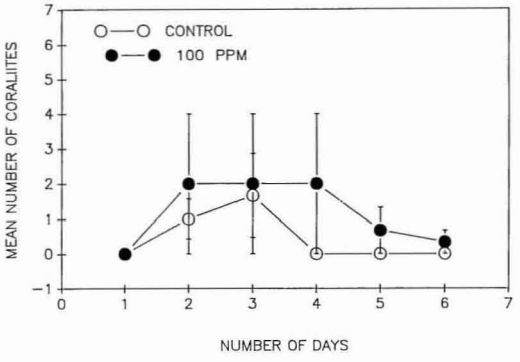

FIGURE 9. Effect of benzene on planulae kept in a closed system with plates (control and 100 ppm).

TABLE 4

EFFECT OF BENZENE ON *Pocillopora damicornis* LARVAE IN 200-ml CONTAINERS WITH SETTLING PLATES HELD IN A CLOSED SYSTEM

FACTOR	MEAN SQUARE	df	*P*
Number of corallites	645.34	1, 10	0.0005
Between treatments	89.90	4, 10	0.0494
Between days	33.42	5, 50	0.0011
Error	25.72		

CLOSED SYSTEM WITH SETTLING PLATES: Figures 7, 8, and 9 show the number of corallites formed after 6 days of exposure. There were more corallites formed in this set with plates in comparison to the previous one without plates (average of 6 versus 1.5; Figures 5–9). Treatment and time effects were found to be significantly different but variability was high (Table 4).

DISCUSSION

Bioassay with Gasoline:Oil Mixture

The open system showed a delay in corallite formation as compared with the closed system (Figures 2–4). This difference in settlement response can be influenced by the coating of the petri dishes by oil globules in the gasoline: oil mixtures. As such, the planulae may have been prevented from sensing their surroundings. Another plausible reason may be the fouling or disorientation of the planulae's sensory systems (Malins et al. 1977, Malins 1979). However, clear correlations between concentration of the test compound and settlement rate was not evident. This condition may have been influenced by the difference in water-soluble fractions of the test compound in the seawater used for the bioassay. Even though the test solutions were premixed and randomly placed into the containers, the actual concentration per container may have varied significantly from one another (MacAulliffe 1977, Rice et al. 1977). Difficulty in determining the exact state of the planulae as exposure time progressed was encountered in several of the closed containers because of "fogging" of the glass containers and formation of slime aggregations inside the bioassay bottles. Nevertheless, the corallites formed were quite visible and were significantly influenced by the test compounds (Tables 1 and 2).

Bioassay with Benzene

The closed system with settling plates had a higher mean number of corallites than the system without plates (Figures 5–9). This implies that settling plates may have influenced the settling response of the planulae when exposed to the hydrocarbon compound. However, variability was quite high for the set with settling plates as compared with the set without plates (Tables 3 and 4). One contributing factor may have been the occurrence of polyp bail-outs and reversed metamorphosis (Richmond 1985) among the previously settled planulae. Another point to consider is the natural variability in settling patterns of the coral planulae (R. A. Kinzie, pers. comm.). This imprecise settling behavior has yet to be fully understood.

In retrospect, the settling response of coral planulae from *P. damicornis* may be unsuitable as a bioassay for the two hydrocarbons used as test compounds. Other biological responses, such as changes in respiration, photosynthetic rate, or both, may offer more substantial bioassay results. In addition, since mortality was not readily observed in most of the experiments, *P. damicornis* planulae may

be affected in other ways not easily detected by visual inspection.

Future Research

The current scarcity of information regarding toxicity and lethality of petroleum products on coral larvae in particular has impeded the scope of this endeavor. The present study can be considered as a preliminary attempt to find the range of toxicity levels and the type of biological reactions exhibited by the coral planulae to the petroleum products. Future studies can further define the precise levels of hydrocarbon compounds in seawater at which the coral planulae are most sensitive. The use of planulae from other coral species may shed more light on the actual response of coral larvae to foreign substances. In addition, the effects of pollutants on the fertilization process, embryonic development, and larval growth of corals may offer more sensitive measures to monitor pollutant levels. Furthermore, sublethal effects may be better monitored through the use of respirometry and the determination of the photosynthesis-irradiance relationship for exposed and control planulae. Last, the quantitative measurements of the actual water-soluble fraction of the test compound in seawater before, during, and after the experiment can yield better correlations regarding settling rate and test compound levels. This may further enhance knowledge on the breakdown and residency time of the hydrocarbon components in seawater and contribute to a more general understanding of how coral larvae react to chemical pollution.

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